

I claim:

1. A method of identifying an alternatively spliced RNA molecule in conjunction with a normally spliced counterpart RNA molecule, comprising the steps of:

(1) obtaining a first population of cDNA molecules from a biological sample representing a first physiological condition and a second population of cDNA molecules from a biological sample representing an second physiological condition;

(2) attaching a first selectable tag to cDNA molecules of the first cDNA population and a second selectable tag to cDNA molecules of the second cDNA population, wherein the first and second selectable tags are different;

(3) denaturing and annealing cDNA molecules from both the first and second cDNA populations, to obtain a mixed population of cDNA molecules;

(4) isolating double-stranded cDNA from the mixed population, wherein the double-stranded cDNA comprises the first and second selectable tags, and also comprises a cDNA molecule from the first cDNA population and a cDNA molecule from the second cDNA population;

(5) selecting from the cDNA isolated in step (4) double-stranded cDNA which comprises at least one region of single-stranded nucleic acid;

(6) coupling both strands of each double-stranded cDNA from step (5) to each other to obtain a coupled molecule; and

(7) comparing both strands of the coupled molecule,

wherein one strand of the coupled molecule represents the alternatively spliced RNA molecule, and the other strand represents the normally spliced counterpart RNA molecule.

2. The method of claim 1, wherein the first biological sample comprises normal tissue, and the second biological samples comprises diseased tissue.

3. The method of claim 1, wherein the first and second biological samples comprise tissue in different developmental states.

4. The method of claim 1, wherein the first biological sample comprises untreated tissue, and the second biological sample comprises tissue which has been treated with a therapeutic or toxic agent.

5. The first and second biological samples can also comprise tissue or cells from different species.

6. The method of claim 1, wherein the first and second biological samples are derived from a human.

7. The method of claim 2, wherein the second biological sample comprises tumor or neoplastic tissue.

8. The method of claim 7, wherein the tumor or neoplastic tissue is derived from a subject with acute promyelocytic leukemia; acute lymphoblastic leukemia; myeloblastic leukemia; uterine cancer; thyroid cancer; gastrointestinal tumors; dysplastic and neoplastic cervical epithelium; melanoma; breast cancer;

prostate cancer; lung cancer; endometrial cancer; teratocarcinoma; colon cancer; brain and desmoplastic round cell tumors; epithelial neoplasias; gastric cancer; ovarian cancer or sarcomas, myomas, myxomas, ependymomas, fibromas, neurofibrosarcomas.

9. The method of claim 2, wherein the second biological sample comprises diseased tissue derived from a subject with infection, stress, disorders or conditions of the immune system; a metabolic disorder; a collagen disorder; a psychiatric disorder, a skin disorder, a liver disorder, a disorders of the arteries; an inherited red cell membrane disorder; thyroid hormone repression; endometrial hyperplasia; Alzheimer's disease; or alcoholism.

10. The method of claim 1, wherein the first and second cDNA populations are synthesized from RNA populations which have been enriched for polyA+ RNA.

11. The method of claim 1, wherein at least one cDNA population comprises double-stranded cDNA.

12. The method of claim 1, wherein the first and second cDNA populations comprise double-stranded cDNA.

13. The method of claim 1, wherein the first and second selectable tags are selected from the group consisting of: biotin; avidin; streptavidin; antigens; haptens; antibodies; hormones; vitamins; receptors; carbohydrates; lectins; metals; chelators; polynucleotides; cofactor or prosthetic groups; apoproteins; effector molecules; one member of a hydrophobic interactive pair; enzyme cofactors; enzymes; polymeric acids; polymeric bases; dyes; protein binders; peptides; protein binders; and enzyme inhibitors, provided that the first and second selectable tags are different.

-35-

14. The method of claim 1, wherein the first selectable tag comprises a biotin.

15. The method of claim 1, wherein the second selectable tag comprises a biotin.

16. The method of claim 1, wherein the first selectable tag comprises a polynucleotide.

17. The method of claim 1, wherein the second selectable tag comprises a polynucleotide.

18. The method of claim 16, wherein the polynucleotide comprises a restriction enzyme target site.

19. The method of claim 17, wherein the polynucleotide comprises a restriction enzyme target site.

20. The method of claim 1, wherein:

1) the first selectable tag comprises an oligonucleotide having a longer and a shorter strand each with a 5' end, that when annealed form a six base pair double-stranded region and an 11 base 5' single-stranded overhang, and wherein a biotin molecule is attached to the 5' end of the longer oligonucleotide strand and the 5' end of shorter oligonucleotide strand is phosphorylated at the 5' end, and wherein the 11 base 5' overhang comprises a six base nucleotide sequence which, when annealed with a single-stranded oligonucleotide comprising the complementary sequence, forms a Sma I restriction site; and

2) the second selectable tag comprises an oligonucleotide having a longer and a shorter strand each with a 5' end, that when annealed form a six

base pair double-stranded region and an 21 base 5' single-stranded overhang, and wherein the 5' end of shorter oligonucleotide strand is phosphorylated at the 5' end, and wherein the 21 base 5' overhang comprises a six base nucleotide sequence which, when annealed with a single-stranded oligonucleotide comprising a complementary sequence, forms a Pml I restriction site.

21. The method of claim 1, wherein in step (3) the cDNA molecules in the first and second cDNA populations are denatured separately, mixed, and annealed to obtain the mixed population of cDNA molecules.

22. The method of claim 1, wherein in step (3) the cDNA molecules in the first and second cDNA populations are mixed together, denatured, and annealed to obtain the mixed population of cDNA molecules.

23. The method of claim 1, wherein an excess of cDNA from one cDNA population relative to the other is used to obtain the mixed population of cDNA molecules.

24. The method of claim 2, wherein an excess of cDNA molecules from the first cDNA population relative to cDNA molecules from the second cDNA population is used to obtain the mixed population of cDNA molecules.

25. The method of claim 24, wherein a 20-fold excess of cDNA from the first cDNA population relative to cDNA molecules from the second cDNA population is used to obtain the mixed population of cDNA molecules.

26. The method of claim 1, wherein step (4) comprises:

(i) selecting molecules comprising the first selectable tag from the mixed population to obtain a first selected population; and

-37-

(ii) selecting molecules comprising the second selectable tag from the first selected population to obtain a second selected population,

wherein the second selected population comprises the mixed population double-stranded cDNA comprising a cDNA molecule from the first cDNA population and a cDNA molecule from the second cDNA population.

27. The method of claim 1, wherein step (4) comprises:

(i) selecting molecules comprising the second selectable tag from the mixed population to obtain a first selected population; and

(ii) selecting molecules comprising the first selectable tag from the first selected population to obtain a second selected population,

wherein the second selected population comprises double-stranded cDNA comprising the first and second selectable tags, and also comprises a cDNA molecule from the first cDNA population and a cDNA molecule from the second cDNA population.

28. The method of claim 1, wherein step (4) comprises contacting the mixed population with an affinity medium.

29. The method of claim 28, wherein the affinity medium comprises a compound selected from the group consisting of: biotin; avidin; streptavidin; antigens; haptens; antibodies; hormones; vitamins; receptors; carbohydrates; lectins; metals; chelators; polynucleotides; cofactor or prosthetic groups; apoproteins; effector molecules; one member of a hydrophobic interactive pair;

enzyme cofactors; enzymes; polymeric acids; polymeric bases; dyes; protein binders; peptides; protein binders; and enzyme inhibitors

30. The method of claim 28, wherein the affinity medium comprises an affinity column.

31. The method of claim 28, wherein the affinity media comprises a solid carrier.

32. The method of claim 31, wherein the solid carrier is selected from the group consisting of: cellulose and cellulose derivatives; polyacrylamide; polystyrenes; polysaccharides; rubber; glass; nylon; polyacrylate; polyvinyltoluene; styrenebutadiamine copolymers; polyacrolein; polyurethane; poly (methyl methacrylate); and combinations thereof.

33. The method of claim 28, wherein the affinity medium comprises a magnetic particle.

34. The method of claim 1, wherein step (5) comprises contacting the double-stranded cDNA from step (4) with a reagent which binds regions of single-stranded DNA.

35. The method of claim 34, wherein the reagent which binds to regions of single-stranded DNA is selected from the group consisting of a resin which binds single stranded DNA, *E. coli* single-stranded binding protein; antibodies which bind to single-stranded DNA; and enzymes which bind to single-stranded DNA.

36. The method of claim 34, wherein the reagent which binds regions of single-stranded DNA is contained in an affinity column.

37. The method of claim 1, wherein step (6) comprises covalently linking both strands of each double-stranded cDNA from step (5) to each other to obtain a coupled molecule.

38. The method of claim 37, wherein both strands of each double-stranded cDNA from step (5) are covalently linked to each other with a polynucleotide linking moiety.

39. The method of claim 38, wherein the polynucleotide linking moiety comprises SEQ ID NO: 5.

40. The method of claim 1, wherein step (7) comprises determining at least a partial nucleotide sequence for each strand of the coupled molecule.

41. A kit for identifying an alternatively spliced RNA molecule in conjunction with a normally spliced counterpart RNA molecule, comprising at least two different selectable tags and their corresponding affinity media, a single-stranded DNA binding reagent, and a linking moiety.

42. A selectable tag comprising an oligonucleotide having a longer and a shorter strand each with a 5' end, that when annealed form a six base pair double-stranded region and an 11 base 5' single-stranded overhang, and wherein a biotin molecule is attached to the 5' end of the longer oligonucleotide strand and the 5' end of shorter oligonucleotide strand is phosphorylated at the 5' end, and wherein the 11 base 5' overhang comprises a six base nucleotide sequence which, when annealed with a single-stranded oligonucleotide comprising the complementary sequence, forms a Sma I restriction site.

43. A selectable tag comprising an oligonucleotide having a longer and a shorter strand each with a 5' end, that when annealed form a six base pair double-stranded region and an 21 base pair 5' single-stranded overhang, and

-40-

wherein the 5' end of shorter oligonucleotide strand is phosphorylated at the 5' end, and wherein the 21 base pair 5' overhang comprises a six base nucleotide sequence which, when annealed with a single-stranded oligonucleotide comprising a complementary sequence, forms a Pml I restriction site.

44. A linking moiety comprising SEQ ID NO: 5.